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On Jan. 13, 2004

TOWNSEND and TOWNSEND and CREW LLP

By: _____

PATENT
020015-000110US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: Q. Nguyen
Buechler et al.	Art Unit: 1636
Application No.: 09/453,234	AMENDED APPEAL BRIEF
Filed: December 1, 1999.	
For: HUMAN ANTIBODIES	

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Sir:

Further to the notice of appeal filed October 8, 2003, this amended brief is submitted in appeal of the final rejection mailed April 9, 2003 in the above-captioned case. This amended brief replaces the original brief filed January 8, 2004. The original brief as noted to have an error in the listing of claims.

I. REAL PARTY IN INTEREST

Biosite Inc., and GenPharm International, Inc., a wholly owned subsidiary of Medarex, Inc.

II. RELATED APPEALS AND INTERFERENCES

None.

III. STATUS OF CLAIMS

Claims 1-35 are pending. All pending claims are rejected and appealed. The claims are listed in Appendix A. The listing of claims assumes the amendment after final will be entered.

IV. STATUS OF AMENDMENTS

An amendment after final is submitted herewith incorporating the limitations of claim 46 into independent claims 1 and 17, and canceling claims 35, 36, 38, and 40-44.

V. SUMMARY OF THE PRESENTLY CLAIMED INVENTION

The presently claimed invention is directed to methods of producing a human antibody display library. The methods entail providing a transgenic mouse whose genome comprises a plurality of human immunoglobulin genes that can be expressed to produce a plurality of human antibodies. A population of nucleic acids encoding human antibody chains is isolated from lymphatic cells of the transgenic mouse forming a library of display packages displaying antibody chains (see specification at pp. 17-19). The libraries comprise at least 100 members at least 50% of which encode human antibodies with an affinity of 10^9 M^{-1} for the same target. No antibody constitutes more than 50% of the library, meaning that the libraries contain a high proportion of diverse high affinity antibodies (see e.g., paragraph spanning pp. 30-31 of the application, table showing affinities in that the 10^{10}-10^{11} range, and p. 65, lines 19-29). The claims specify that the transgenic mice used in the methods comprise less than the full complement of human immunoglobulin genes (see e.g., p. 11, lines 4-22 and at p. 36, lines 11-21). The claims also specify that the isolation of nucleic acids from transgenic mice is performed using a set of primers selected based on which human immunoglobulin genes from the full complement of human immunoglobulin genes are present in the genome of the mouse (i.e., customized primers) (see, e.g., specification at p. 42, lines 8-31 and in Tables A, B, and C at p. 43).

The use of a transgenic mouse incorporating less than a full complement of human immunoglobulin genes is believed to be advantageous in generating populations of high affinity antibodies for the reasons discussed in the specification at p. 11, lines 4-22 and at p. 36, lines 11-21. In brief, the more limited complement of human immunoglobulin genes present in such animals result in a reduced proportion of unnatural random permutations of heavy and light

chains incapable of high affinity binding. Use of a primer set customized to the human immunoglobulin genes from the full complement of human immunoglobulin genes is also advantageous in reducing mutations incorporated into amplified sequences and/or reducing failed amplification due to primer mismatch, as discussed in more detail below.

VI. ISSUE

Whether claims 1-36, 38, and 40-44 would have been obvious under 35 USC §103(a) over Gray (WO9847343) or Buechler (US 6,057,098) in view of Kucherlapati (WO 96/33735) and Lonberg (US 5,770,429).

VII. GROUPING OF THE CLAIMS

The claims do not stand or fall together. At least claims 40 and 44 are distinguished from the cited references on additional grounds as discussed in more detail below.

VIII. ARGUMENT

1. The Examiner's rationale

Gray and Buechler are cited as discussing methods of phage display that achieve populations of antibodies with high affinities (final office action at pp. 3-4). The Examiner acknowledges that Gray and Buechler do not disclose producing a human antibody display library using populations of nucleic acids encoding human immunoglobulins from transgenic mice (final office action at p. 4, second paragraph). Kucherlapati and Lonberg are cited as disclosing transgenic animals expressing human immunoglobulins (final office action at pp. 5-6). Kucherlapati is further cited as teaching combination of phage display technology with such a transgenic animal (final office action at p. 7). The Examiner takes the view it would have been obvious to combine the references for the benefit of producing high affinity antibodies (final office action at p. 7). With respect to previous claim 46 (whose elements are now included in all independent claims), the Examiner takes the view that it would have been obvious and within the skill of the art of the artisan at the effective filing date of the present application to design a set of primers selected based on which human immunoglobulin genes are present in the genome of the transgenic mouse (final office action at pp. 7-8). The Examiner bases this view on the high level of skill in the art, and the teachings of Gray that libraries of high affinity antibodies can be obtained without the use of customized primers (final office action at p. 8).

2. Summary of the Cited Art

Kucherlapati discusses transgenic mice encoding human immunoglobulin genes. All of the examples and most of the general description are directed to isolation of human antibodies from such mice via hybridoma technology (see e.g., pp. 20-31). That is B-cells from such mice are fused with lymphomas to generate hybridomas which secrete human antibodies. Kucherlapati does briefly and prophetically discuss an alternative means of isolating antibodies from such mice using phage display (see pp. 11-12). In this discussion, Kucherlapati does not provide any indication that modifications to previous phage display protocols might be desirable to adapt phage display to use in combination with a transgenic mouse. Thus, Kucherlapati teaches that antibody chains be amplified using the primer set previously used by Marks et al., *J. Mol. Biol. 581-596* (1991) (of record) (see Kucherlapati at p. 13, lines 7-9). The Marks reference uses phage display to screen antibody sequences from an unimmunized human. Marks' primer sets contain far fewer primers than there are natural immunoglobulin genes. Therefore, Marks probably selected his primers either from certain representative immunoglobulin sequences or from consensus sequences of different immunoglobulins. In any event, Mark's primers were not selected based on the subset of human immunoglobulins that are present in a particular transgenic mouse, as specified in the present claims.

Lonberg also discusses transgenic mice encoding human immunoglobulin chains. The reference does not discuss primers for use in cloning populations of nucleic acids encoding human antibodies from transgenic mice.

Buechler and Gray provide essentially the same disclosure relating to improved methods of phage display for isolating populations of high affinity antibodies. The examples in these patents relate to obtaining populations of antibodies from normal (i.e., nontransgenic mice). There is no discussion of primers for cloning populations of nucleic acids encoding human antibodies from transgenic mice.

3. The Prior Art Does Not Teach All Claim Limitations

The prior art references when combined must teach or suggest all of the claim limitations. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Here, even assuming

arguendo that the cited references are properly combined, the references neither individually or in combination provide any disclosure of customized primers, as claimed. The only reference providing any discussion of primers for cloning populations of human antibody genes from transgenic mice is Kucherlapati. The only such primers discussed in this reference are noncustomized primers discussed by Marks. These primers are not designed with respect to a subset of human immunoglobulin genes present in transgenic mice. Rather, these are intended for amplification of immunoglobulin sequences present in an immunized natural human.

The difference between using a primer set containing primers customized to amplify the human immunoglobulin sequences present in a transgenic mouse, and a primer set intended for amplification of immunoglobulin sequences present in an unimmunized natural human is illustrated by the attached two figures (previously of record). The upper part of Figure 1 shows the amino acid sequence from the N-terminus of human heavy chains isolated using customized VH primers as exemplified in the present specification at p.43 (these primers are designated as the Biosite/Medarex primers in the Figures). The sequences designated M1- or M2- are the same as corresponding sequences shows at pp. 84-87 in the present application. The sequences designated 1C- or 3E- are described in commonly owned related application PCT US 00/27237. The lower part of Fig. 1 shows the amino acids encoded by Marks' primer compared with the primers disclosed in the present application. The column labeled "hits" indicates how many of the antibody sequences are encoded by a particular primer. Twenty-nine of thirty-one heavy chain sequences are encoded by one of the primers disclosed in the present application. By contrast, only 13 of the 31 heavy chain sequences are encoded by one of Marks' primers. Use of Marks' primers to attempt to amplify other than these 13 immunoglobulin sequences would either not result in amplification due to lack of complementarity or would result in introduction of mutations. Fig. 2 presents similar data for light chain sequences. In this case, Marks primers encode only 11 of the 31 sequences. Accordingly, use of Marks primer set would result in loss or mutation of a substantial number of antibodies that are obtained using a primer set customized to the human immunoglobulin genes present in a transgenic mouse.

Thus, the selection of customized primers can have a significant effect on the libraries generated by the claimed methods. The prior art does not teach this claim limitation.

4. No Motivation to Modify Cited Art

It is undisputed that the cited art does not teach the claim limitation of customized primers, as discussed above. The sole remaining issue is whether sufficient motivation to modify the cited art has been identified. The motivation must have sufficient "force" to "impel persons skilled in the art to do what applicant has done." *Ex parte Levengood,* 28 USPQ2d 1300, 1302 (BPAI 1993). "Actual evidence" of "clear and particular" motivation is required. *In re Dembiczak*, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999). "Broad conclusory statements regarding the teaching of multiple references" are insufficient. The requirement for evidence of particularized motivation provides a safeguard against the "tempting but forbidden zone of hindsight." *Dembiczak* at p. 1616. Here, several potential sources of motivation asserted by the Examiner to alter the teaching of the cited references will be considered in turn.

The first alleged source of motivation is that "applicants failed to provide any objective evidence for why one of ordinary skilled artisan would limit exclusively the teachings of Kucherlapati et al. with the use of Marks' set of primers" (office action of November 12, 2002 at sentence bridging pp. 14-15). The Examiner also says that Kucherlapati does not teach the exclusive use of Marks' primer set, such as the mouse of Lonberg (final office action at p. 12). Insofar as the Examiner looks to appellants to identify negative teaching in the reference regarding using primers other than Marks, he is incorrectly transferring the PTO's burden of proof to appellants. In proceedings before the Patent and Trademark Office, the examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art (*In re Piasecki*, 223 USPQ 785, 787-88 (Fed. Cir. 1984)). If the evidence is in "equipoise," an inventor is "entitled to a patent." *In re Oetiker,* 24 USPQ2d 1443, 1447 (Fed. Cir. 1992) (Plager, J., concurring). Although teaching away evidence may be sufficient for patentability, it is not necessary. The burden is on the Examiner to show that the reference discloses or suggests use of the customized primer sets specified in the pending claims, not for appellants to identify negative or teaching away evidence. Here, Marks' primer sets are the only primers mentioned by Kucherlapati. Kucherlapati does not provide any reason that one would want to consider any other primers, either with his own mice or with any other, such as that of Lonberg. Kucherlapati provides no indication even that this issue is worthy of further consideration. In these circumstances, pointing to lack of teaching away evidence does not fulfill the PTO's burden of

providing actual evidence of clear and particular motivation to modify the Kucherlapati's teaching to select Marks's primers.

Next the Examiner alleges that it "would have been obvious and within the level of skill for an ordinary artisan to devise an appropriate customized primer set for PCR amplifying the genes encoding high-affinity antibodies depending on which transgenic mouse being used." The Examiner adds the level of skill in the art is high and that the artisan can think. Final office action at p. 12. However, "[t]hat which is within the capabilities of one skilled in the art is not synonymous with obviousness." *Ex parte Gerlach,* 212 USPQ 471 (Bd.App. 1980). An "assertion that one of ordinary skill in the relevant art would have been able to arrive at applicant's invention because he had the necessary skills to carry out the requisite process steps" is an "inappropriate standard for obviousness." *Orthokinetics Inc. vs. Safety Travel Chairs Inc.*, 1 USPQ2d 1081 (Fed. Cir. 1986). "The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggested the desirability of the modification." *In re Fritch,* 23 USPQ2d 1780, 1784 (Fed. Cir. 1992). Here, the cited art does not suggest the desirability of the modification. The desirability is only apparent from the type of analysis discussed in section VIII 3. above. Kucherlapati provides only a brief and prophetic discussion of combining phage display and transgenic mice. The reference directs the reader to a set of primers that had been used previously for amplifying human immunoglobulin libraries for phage display. He provides no reason to look to other primers or even any indication that this issue was worthy of further consideration. Although, as the Examiner says, the skilled artisan can think, he is "*presumed to be one who thinks along the lines of conventional wisdom in the art....*" *Standard Oil Co. vs. American Cyanamid Co.*,227 USPQ 293, 398 (Fed. Cir. 1985), at p. 454 (emphasis supplied). Kucherlapti's teaching to use the primers of Marks, which were conventional in the art, would merely have reinforced this mindset.

Next the Examiner points to Gray or Buechler as demonstrating feasibility of obtaining libraries of high affinity antibodies without use of customized primers (final office action at p. 12). Initially, it is noted that Gray and Buechler amplified libraries of mouse antibodies from nontransgenic mice, and do not specify primers for use in cloning human antibodies from a transgenic mouse. Insofar as Gray or Buechler achieved libraries with high

affinities without use of customized primers, and such teaching was thought relevant to producing libraries of human antibodies from transgenic mice, then such disclosure would teach away from rather than toward the use of customized primers. If one thought that previously used primer sets could not be improved on, one would not have been motivated to consider different strategies of primer design.

Finally, the Examiner says the claims do not recite any characteristics of the customized primers that yield unexpected results as asserted by appellants. However, appellants' position is not dependent on a showing of unexpected results. Rather, appellants' position is that that the Examiner has not made a prima facie case showing that the prior art references when combined teach or suggest all of the claim limitations. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

Further, it is submitted that the cited references did not provide a reasonable expectation of success of obtaining the population of at least 100 human antibodies at least 50% of which have an affinity of at least 10^9 M^{-1}, as recited in claim 1, and particularly not the libraries of at least 100 human antibodies at least 50% of which have an affinity of at least 10^{10} M^{-1} recited in claims 40 and 44. The prior art cannot be modified or combined to reject claims as prima facie obvious without a reasonable expectation of success. *In re Merck & Co, Inc.*, 231 USPQ 375, 379 (Fed. Cir. 1986). Here, the feasibility of generating the claimed libraries is evidenced by the Table at p. 75 showing affinities in that the 10^{10}-10^{11} M^{-1} range, and p. 65, lines 19-29 showing that a high proportion of antibodies that were tested have such affinities. The success of the presently claimed methods in providing virtually unlimited numbers of high affinity human antibodies was not reasonably expected viewed from the perspective that generation of human antibodies with high affinity has long been regarded as a difficult task.

For most of the prosecution up to the final office action, the Examiner's principal basis that the references provide a reasonable expectation of success has been Kucherlapati's alleged teaching that combination of the Xenomouse with phage display offers significant advantage over previous applications of phage display (see e.g., final office action at sentence bridging pp. 5-6, office action of November 12, 2002 at p. 13, second paragraph)). However, the Examiner has taken this statement out of context. Although Kucherlapati does indicate that combination of phage display with the Xenomouse may be advantageous over previous

applications of phage display, the advantage he identifies is that of extending the application of phage display to generation of human antibodies to human antigens (pp. 12-13).

As Kucherlapati explains, phage display has been successfully used by others such as Burton et al. to generate moderate affinity antibodies (ca. 10^8 M^{-1}) to nonhuman antigens such as HIV, but has been much less successful in generating human antibodies to human antigens because of the inability to use such antigens as immunogens in a human. According to Kucherlapati, use of the Xenomouse would allow one to immunize with a human antigen, and thereby presumably use phage display to obtain human antibodies to the human antigen in similar fashion to that employed by Burton to generate human antibodies to HIV. Kucherlapati does not say, however, that combination of the Xenomouse with phage display would allow one to generate higher affinity human antibodies to a human antigen than one could generate to a nonhuman antigen without a Xenomouse, such as described by Burton. The Examiner is thus taking Kucherlapati's comment on the advantage of combining phage display with the Xenomouse out of the context in which it was made, and overgeneralizing it into a general advantage of antibodies prepared using a combination of phage display and the Xenomouse to antibodies made using the Xenomouse alone.

The Examiner also refers to Table 4 of Kucherlapati apparently as disclosing examples of the high affinity human antibodies that might be generated by the combination of phage display and a transgenic mouse (office action of November 12, 2002 at p. 13, second paragraph). However, the Examiner ignores the fact that the antibodies referred to in Table 4 were not generated by a combination of phage display and a transgenic mouse but using a transgenic mouse alone. As discussed in the last response, the natural pairings of heavy and light chain which are represented in antibodies isolated directly from a Xenomouse are likely to be lost during phage display. Thus, antibodies isolated using a combination of phage display and a Xenomouse would not necessarily be expected to have similar affinities to those isolated from the Xenomouse directly. In addition, Kucherlapati provides no indication of how many antibodies he had to screen to obtain the few high affinity antibodies shown in Table 4 of Kucherlapati. Thus, it is not at all apparent that Kucherlapati was able to isolate high affinity human antibodies at high frequency directly from the Xenomouse in contrast to the presently claimed methods.

For these reasons, it is not reasonably predictable from the Kurcherlapati reference that one could combine phage display and a transgenic mouse to generate the claimed libraries displaying large populations of high affinity human antibodies.

The Examiner also refers to a claim in Lonberg as specifying a human antibody produced from a transgenic mouse with an affinity of 10^{10} M^{-1} (final office action at p. 6, second paragraph) However, it is not disputed that antibodies having such affinities can be produced directly from transgenic mice. What is at issue is the frequency of representation of such antibodies both as directly isolated from a transgenic mouse, and when isolated by combining phage display with a transgenic mouse when the additional variable of random assortment of heavy and light chains occurs. As was noted in connection with Table 4 of Kucherlapati, Kucherlapati does not indicate the representation of high affinity human antibodies obtainable directly from a transgenic mouse, much less whether this representation is maintained notwithstanding the effects of random assortment of heavy and light chains. The Examiner has not identified what teaching in Lonberg compensates for this deficiency in Kucherlapati.

In the final office action, the Examiner discounts the above arguments on the basis that Gray or Buechler rather than Kucherlapati is the primary reference (final office action at p. 10). The Examiner even questions whether appellants are implying that the patent of Buechler is invalid (final office action at p. 11, second paragraph). In response, appellants certainly do not imply that Buechler's patent is invalid, but merely point out that neither Buechler nor Gray provides data on the affinity of human antibodies that can be isolated from a transgenic mouse using phage display or otherwise. Indeed, the Examiner himself has stated that "regarding to claims directed to a library in which library members encode heavy and light human antibody chains having specific affinity at least $10^{9}M^{-1}$ and $10^{10}M^{-1}$, neither reference [Gray or Buechler] clearly demonstrates that such a library was obtainable" (office action of November 12, 2002 at p. 6, second paragraph). In the circumstances, it is evident, notwithstanding the protestations to the contrary in the final office action, that the Examiner is relying primarily on Kucherlapati for establish a reasonable expectation of success. Kucherlapati does not do so for the reasons discussed above.

IX. CONCLUSION

The Examiner has not identified actual evidence of clear and particular motivation to modify the teachings of the cited references to use a set of primers selected based on which human immunoglobulin genes from the full complement of human immunoglobulin genes are present in a transgenic mouse. Alleged lack of teaching away evidence in Kucherlapati, the level of skill in the art, and broad conclusory statements regarding the other cited references cannot compensate for the absence of an evidentiary source of particularized motivation. Absent the safeguard provided by an evidentiary source of particularized motivation, one has no way of knowing that the mental reconstruction needed by the Examiner to obtain the claimed invention was not the result of hindsight. Moreover, the Examiner has not established the cited art provided a reasonable expectation of success. For these reasons, it is respectfully submitted that the outstanding rejection should be reversed.

Respectfully submitted,

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APPENDIX A: PENDING CLAIMS

1. A method of producing a human antibody display library, comprising:

providing a transgenic mouse whose genome comprises a plurality of human immunoglobulin genes that can be expressed to produce a plurality of human antibodies, wherein the transgenic mouse comprises less than the full complement of human immunoglobulin genes present in a human being;

isolating a population of nucleic acids encoding human antibody chains from lymphatic cells of the transgenic mouse by amplifying the population of nucleic acids using a set of primers selected based on which human immunoglobulin genes from the full complement of human immunoglobulin genes are present in the genome of the transgenic mouse;

forming a library of display packages displaying the antibody chains, wherein a library member comprises a nucleic acid encoding an antibody chain, and the antibody chain is displayed from the package, wherein the library comprises at least 100 members at least 50% of which comprise nucleic acids encoding human antibody chains showing at least 10^9 M^{-1} affinity for the same target and no library member constitutes more than 50% of the library.

2. The method of claim 1, further comprising producing RNA transcripts of the nucleic acids, and translating the transcripts to form antibody chains under conditions in which an antibody chain remains linked to the RNA transcript from which the antibody chain was translated, the complex formed between the transcript and the antibody chain constituting a library member.

3. The method of claim 1, further comprising cloning the population of nucleic acids into multiple copies of a phage display vector and expressing the vector in host cells to form the library of display packages.

4. The method of claim1, wherein the display package comprises a phagemid vector.

5. The method of claim 1, wherein the nucleic acids encode variable regions of the antibody chains and the display package comprises a segment encoding a human constant region

and the cloning joins a nucleic acid encoding a variable region in-frame with the segment encoding the human constant region.

6. The method of claim 5, wherein the antibody chain is a heavy chain and the constant region comprises a C_H1 region.

7. The method of claim 5, wherein the antibody chain is a light chain and the constant region comprises a C_κ or C_λ constant region.

8. The method of claim1, wherein the antibody chain comprises a heavy or light chain which in at least some library members is complexed to a binding partner, comprising respectively a partner light or heavy human chain to form a Fab fragment.

9. The method of claim 1, further comprising contacting libraries members with a target, whereby library members displaying an antibody chain and binding partner (if present) with specific affinity for the target bind to the target, and separating display packages displaying antibody chains bound to the target to produce a subpopulation of display packages.

10. The method of claim 9, further comprising immunizing the transgenic mouse with an antigen.

11. The method of claim 10, wherein the antigen is the target or an immunogenic fragment thereof.

12. The method of claim 1, wherein a library member further comprises a nucleic acid segment encoding a tag linked to the nucleic acid encoding the antibody chain, wherein the tag is the same in different library members.

13. The method of claim 12, further comprising contacting library members with a receptor having specific affinity for the tag and isolating a subpopulation of library members that bind to immobilized receptor.

14. The method of claim 13, further comprising contacting the subpopulation of library members with a target lacking specific affinity for the tag, and isolating a further subpopulation of library members that binds to the target.

15. The method of claim 14, further comprising subcloning en masse nucleic acids encoding antibody chains from the further subpopulation of library members into multiple copies of an expression vector to form modified expression vectors.

16. The method of claim 15, further comprising expressing the modified expression vectors in host cells to produce a library of human antibody chains.

17. A method of producing a human Fab phage display library, comprising:

providing a transgenic mouse whose genome comprises a plurality of human immunoglobulin genes that can be expressed to produce a plurality of human antibodies, wherein the transgenic mouse comprises less than the full complement of human immunoglobulin genes present in a human being;

isolating populations of nucleic acids respectively encoding human antibody heavy chains and human antibody light chains from lymphatic cells of the transgenic mouse by amplifying the populations of nucleic acids using a set of primers selected based on which human immunoglobulin genes from the full complement of human immunoglobulin genes are present in the genome of the transgenic mouse;

cloning the populations into multiple copies of a phage display vector to produce a display library, wherein a library member comprises a phage capable of displaying from its outersurface a fusion protein comprising a phage coat protein, a human antibody light chain or human antibody heavy chain, wherein in at least some members, the human antibody heavy or light chain is complexed with a partner human antibody heavy or light chain, the complex forming a Fab fragment to be screened, wherein the library comprises at least 100 members at least 50% of which comprise nucleic acids encoding Fab fragments showing at least 10^9 M^{-1} affinity for the same target and no library member constitutes more than 50% of the library.

18. The method of claim 17, wherein the plurality of human genes is free of human lambda light chain genes.

19. The method of claim 17, wherein there are no more than 40 human VH genes included in the plurality of human genes.

20. The method of claim 17, wherein there are no more than 40 human VL genes included in the plurality of human genes.

21. The method of claim 17, wherein each copy of the phage display vector receives a random combination of nucleic acids encoding heavy and light chains from the respective populations.

22. The method of claim 17, wherein the populations of nucleic acids respectively encode populations of human heavy and light chain variable regions, and the phage display vector encodes human heavy and light chain constant regions expressed in frame with human heavy and light chains inserted into the vector.

23. The method of claim 17, further comprising contacting libraries members from the display library with a target, whereby library members displaying a Fab fragment with specific affinity for the target bind to the target, and separating phage displaying Fab fragments bound to the target to produce a further subpopulation of phage.

24. The method of claim 23, further comprising isolating a phage displaying a Fab fragment that binds to the target.

25. The method of claim 17, further comprising immunizing the transgenic mouse with an antigen.

26. The method of claim 24, further comprising expressing a Fab fragment from a phage bound to the target in soluble form.

27. The method of claim 17, wherein the fusion protein further comprises a tag that is the same in different library members.

28. The method of claim 27, further comprising contacting library members with a receptor that specifically binds to the tag, and isolating a subpopulation of library members bound to the receptor.

29. The method of claim 28, further comprising contacting the subpopulation of library members with a target lacking specific affinity for the tag, and isolating a further subpopulation of library members bound to the target.

30. The method of claim 29, further comprising subcloning a mixed population of nucleic acids encoding human antibody heavy chains and human antibody light chains from the further subpopulation of library members into multiple copies of an expression vector to produce modified expression vectors.

31. The method of claim 30, further comprising expressing the modified expression vectors in host cells to produce a population of human antibodies.

32. The method of claim 31, wherein the population of human antibodies includes at least 10 different antibodies.

33. The method of claim 32, wherein the population of human antibodies includes at least 100 different antibodies.

34. The method of claim 33, wherein the population of human antibodies includes at least 1000 different antibodies.

Figure: 1 Compilation of Human Heavy amino termini amplified with the Biosite/Medarex PCR primer set and compared with the Marks' human V_H Back Primers (Marks et.al. 1991).

	1				50	Primer
1CB1H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTLR	SYAMHWVRQA	PGKGLEWVAV	948
1CC2H	EVQLVQSGGG	VVQPGRSLRL	SCAASEFTFS	NYAFHWVRQA	PGKGLEWVAI	946
1CC6H	QVQLVQSGGG	VVQSGRSLRL	SCAASGITVR	NYAMHWVRQV	PGKGLEWVAV	944/1a
1CC8H	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	NYAFHWVRQA	PGKGLEWVAI	944/1a
1CD7H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFS	NYAMHWVRQA	PGKGLEWVAI	948
1CE8	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	NYAFHWVRQA	PGKGLEWVAI	944/1a
3E1H	EVQLVQSGGG	LVQPGGSLRL	SCAASGFTFS	NYAMSWVRQA	PGKGLEWVSA	946
3E2H	QVQLVQSGAE	VKKPGESLKI	SCKGSGYSFT	NYWIGWVRQM	PGKGLEWMGF	944/1a
3E3H	QVQLVQSGAE	VKKPGESLKI	SCKGSGYSFT	NYWIGWVRQM	PGKGLEWMGF	944/1a
3E4H	QVQLVQSGGG	VVQSGRSLRL	SCAASGITVR	NYAMHWVRQV	PGKGLEWVAV	944/1a
3E8H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFR	RYGMHWVRQA	PGKGLEWVAV	948
3E9H	QVQLVQSGAE	VKKPGESLKI	SCKGSGYSFT	NYWIGWVRQM	PGKGLEWMGI	944/1a
M1_10H	QVQLVQSGGG	LVHPGGSLRL	SCEGSGFIFR	NHPIHWVRQA	PGKGLEWVSV	944/1a
M1_1H	QVQLVESGGG	VVQPGKSLRL	SCAASEFTIS	YYGMHWVRQV	PGKGLEWVAA	948
M1_21H	QVQLVQSGGG	VVQPGKSLRL	SCAASGFTFS	YYGMHWVRQV	PGKGLEWVAA	944/1a
M1_23H	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	NYGMHWVRQA	PGKGLEWVAA	944/1a
M1_25H	QVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	YYGMHWVRQV	PGKGLEWVAA	948
M1_3H	DVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVTL	?
M1_4H	QVQLVESGGG	VVQPGKSLRL	SCAASGFTFS	YYGMHWVRQV	PGKGLEWVAA	948
M1_5H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVTL	948
M1_8H	QVQLVQSGGG	VVQPGKSLKL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVAA	944/1a
M2_11H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVTL	948
M2_12H	DVQLVESGGG	VVHPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWMTL	?
M2_16H	QVQLVQSGGG	VVQPGKSLRL	SCAASGFSLS	YYGMHWVRQV	PGKGLEWVAA	944/1a
M2_18H	QVQLVQSGGG	VVQPGKSLRL	SCAASGFSFS	YYGMHWVRQV	PGKGLEWVAA	944/1a
M2_20H	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVSL	944/1a
M2_31H	QVQLVESGGV	VVQPGRSLRL	SCAASGFTFS	YYGIHWVRQV	PGKGLEWVAL	948
M2_32H	QVQLVQSGGG	LVHPGGSLRL	SCEGSGFIFR	NHPIHWVRQA	PGKGLEWVSV	944/1a
M2_33H	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWMTL	944/1a
M2_34H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFS	YYGIHWVRQV	PGKGLEWVVL	948
M2_35H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTIS	YYGIHWVRQV	PGKGLEWVEL	948

Marks Human V_H Back Primers

	Amino Terminus	#Hits
$HuV_H1aBACK$	QVQLVQSG	16
$HuV_H2Aback$	QVNLRESG	0
$HuV_H3Aback$	EVQLVESG	0
$HuV_H4Aback$	QVQLQESG	0
$HuV_H5Aback$	EVQLLQSA	0
$HuV_H6Aback$	QVQLQQSG	0

Biosite/Medarex V_H HuMab Primers

	Amino Terminus	#Hits	
#944	QVQLVQSG	16	1aBACK = #944
#945	EVQLLESG	0	
#188	EVQLVESG	0	3aBACK = #188
#946	EVQLVQSG	2	
#947	QVQLQQWG	0	
#948	QVQLVESG	11	

*Bold primers are unique to either Marks'or Biosite/Medarex

Figure 2: Compilation of Human Kappa amino termini amplified with the Biosite/Medarex PCR primer set and compared with Marks' Human V_K Back Primers

	1				50	Primer
1CB1K	EIVMTQSPAT	LSLSPGERAT	LSCRASQSVY	S.YLVWYQQK	PGQAPRLLIY	935
1CC2K	ELVMTQSPAT	LSLSPGERAT	LSCRASQSVY	S.YLVWYQQK	PGQAPRLLIY	?
1CC6K	EIVMTQSPGT	LSLSPGERAT	LSCRASQSVS	SRYLAWYQQK	PGQAPRLLIY	935
1CC8K	EIVLTQSPGT	LSLSPGERAT	LSCRASQSIY	N.YLAWYQQK	PGQAPRLLIY	189/937/3a/6a
1CD7K	ELVMTQSPAT	LSLSPGERAT	LSCRASQSIY	N.YLAWYQQK	PGQAPRLLIY	?
1CE8K	ELVMTQTPLS	LSLSPGERAT	LSCRASQNVY	S.YLAWYQQK	PGQAPRLLIY	?
3E1K	ELVMTQTPLS	LSLSPGERAT	LSCRASQSIY	N.YLAWYQQK	PGQAPRLLIY	?
3E2K	NIQMTQSPSS	LSASVGDRVT	ITCRASQGIS	S.WLAWYQQK	PEKAPKSLIY	932
3E3K	DIQMIQSPSS	PSASVGDRVT	ITCRASQGIS	S.ALAWYQQK	PGKAPKLLIY	955
3E4K	EIVMTQSPGT	LSLSPGERAT	LSCRASQSVS	SRYLAWYQQK	PGQAPRLLIY	935
3E8K	AIQLTQSPSS	LSASVGDRVT	ITCRASQGIS	S.ALAWYQQK	PEKAPKLLIY	934
3E9K	ELVMTQSPSS	LSASVGDRVT	ITCRASQGIS	S.WLAWYQQK	PEKAPKSLIY	?
M1_10L	DVVMTQSPAT	LSLSPGERAT	LSCRASQSVS	S.YLAWYQQK	PGQAPRLLIY	936/2a
M1_1L	EIVLTQSPAT	LSLSPGERAT	LSCRASQGVS	S.YLAWYQQK	PGQAPRLLIY	189/937/3a/6a
M1_21L	AIRMTQSPSF	LSASVGDRVT	ITCRASQSIS	S.YLNWYQQK	PGKAPKLLIY	933
M1_23L	EIVLTQSPGT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLLIY	189/937/3a/6a
M1_25L	EIVLTQSPGT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLLIY	189/937/3a/6a
M1_3L	EIVMTQSPAT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLLIY	935
M1_4L	EIVLTQSPGT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLHIY	189/937/3a/6a
M1_5L	EIVMTQSPGT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLLIY	935
M1_8L	EIVMTQSPGT	LSLSPGERAT	LSCRASQSVS	STYLAWYQQK	PGQAPRLLIY	935
M2_11L	EIVMTQSPGT	LSLSPGERAT	LSCRASQGVS	SSYLAWYQQK	PGQAPRLLIY	935
M2_12L	EIVMTQSPGT	LSLSPGERAT	LSCRASQGVS	SSYLAWYQQK	PGQAPRLLIY	935
M2_16L	EIVMTQSPGT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLLIY	935
M2_18L	EIVMTQSPGT	LSLSPGERAT	LSCRASQSVS	STYLAWYQQK	PGQAPRLLIY	935
M2_20L	EIVMTQSPGT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLLIY	935
M2_31L	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	S.YLAWYQQK	PGQAPRLLIY	189/937/3a/6a
M2_32L	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	S.YLAWYQQK	PGQAPRLLIY	189/937/3a/6a
M2_33L	EIVLTQSPGT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLLIY	189/937/3a/6a
M2_34L	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	S.YLAWYQQK	PGQAPRLLIY	189/937/3a/6a
M2_35L	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	S.YLAWYQQK	PGQAPRLLIY	189/937/3a/6a

Marks Human V_K Back Primers

	Amino Terminus	#Hits
$HuV_K1aBACK$	DIQMTQSP	0
$HuV_K2Aback$	DVVMTQSP	1
$HuV_K3Aback$	EIVLTQSP	10
$HuV_K4Aback$	DIVMTQSP	0
$HuV_K5Aback$	ETTLTQSP	0
$HuV_K6Aback$	EIVLTQSP	(10)

Biosite/Medarex V_K HuMab Primers

	Amino Terminus	#Hits	
#955	DIQMIQSP	1	
#936	DVVMTQSP	1	(2aBACK=936)
#189/937	EIVLTQSP	10	(3aBACK=189/937)
#931	VIWMTQSP	0	
#932	NIQMTQSP	1	
#937/189	EIVLTQSP	(10)	(6aBACK=937/189)
#933	AIRMTQSP	1	
#934	AIQLTQSP	1	
#935	EIVMTQSP	11	
#956	DIVMTQTP	0	

*Bold primers are unique to either Marks or Biosite/Medarex

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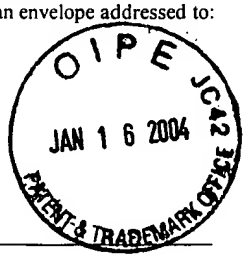

TOWNSEND and TOWNSEND and CREW LLP

By: _____

PATENT
020015-000110US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: Q. Nguyen
Buechler et al.	Art Unit: 1636
Application No.: 09/453,234	AMENDED APPEAL BRIEF
Filed: December 1, 1999.	
For: HUMAN ANTIBODIES	

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Sir:

Further to the notice of appeal filed October 8, 2003, this amended brief is submitted in appeal of the final rejection mailed April 9, 2003 in the above-captioned case. This amended brief replaces the original brief filed January 8, 2004. The original brief as noted to have an error in the listing of claims.

I. REAL PARTY IN INTEREST

Biosite Inc., and GenPharm International, Inc., a wholly owned subsidiary of Medarex, Inc.

II. RELATED APPEALS AND INTERFERENCES

None.

III. STATUS OF CLAIMS

Claims 1-35 are pending. All pending claims are rejected and appealed. The claims are listed in Appendix A. The listing of claims assumes the amendment after final will be entered.

IV. STATUS OF AMENDMENTS

An amendment after final is submitted herewith incorporating the limitations of claim 46 into independent claims 1 and 17, and canceling claims 35, 36, 38, and 40-44.

V. SUMMARY OF THE PRESENTLY CLAIMED INVENTION

The presently claimed invention is directed to methods of producing a human antibody display library. The methods entail providing a transgenic mouse whose genome comprises a plurality of human immunoglobulin genes that can be expressed to produce a plurality of human antibodies. A population of nucleic acids encoding human antibody chains is isolated from lymphatic cells of the transgenic mouse forming a library of display packages displaying antibody chains (see specification at pp. 17-19). The libraries comprise at least 100 members at least 50% of which encode human antibodies with an affinity of 10^9 M^{-1} for the same target. No antibody constitutes more than 50% of the library, meaning that the libraries contain a high proportion of diverse high affinity antibodies (see e.g., paragraph spanning pp. 30-31 of the application, table showing affinities in that the 10^{10}-10^{11} range, and p. 65, lines 19-29). The claims specify that the transgenic mice used in the methods comprise less than the full complement of human immunoglobulin genes (see e.g., p. 11, lines 4-22 and at p. 36, lines 11-21). The claims also specify that the isolation of nucleic acids from transgenic mice is performed using a set of primers selected based on which human immunoglobulin genes from the full complement of human immunoglobulin genes are present in the genome of the mouse (i.e., customized primers) (see, e.g., specification at p. 42, lines 8-31 and in Tables A, B, and C at p. 43).

The use of a transgenic mouse incorporating less than a full complement of human immunoglobulin genes is believed to be advantageous in generating populations of high affinity antibodies for the reasons discussed in the specification at p. 11, lines 4-22 and at p. 36, lines 11-21. In brief, the more limited complement of human immunoglobulin genes present in such animals result in a reduced proportion of unnatural random permutations of heavy and light

chains incapable of high affinity binding. Use of a primer set customized to the human immunoglobulin genes from the full complement of human immunoglobulin genes is also advantageous in reducing mutations incorporated into amplified sequences and/or reducing failed amplification due to primer mismatch, as discussed in more detail below.

VI. ISSUE

Whether claims 1-36, 38, and 40-44 would have been obvious under 35 USC §103(a) over Gray (WO9847343) or Buechler (US 6,057,098) in view of Kucherlapati (WO 96/33735) and Lonberg (US 5,770,429).

VII. GROUPING OF THE CLAIMS

The claims do not stand or fall together. At least claims 40 and 44 are distinguished from the cited references on additional grounds as discussed in more detail below.

VIII. ARGUMENT

1. The Examiner's rationale

Gray and Buechler are cited as discussing methods of phage display that achieve populations of antibodies with high affinities (final office action at pp. 3-4). The Examiner acknowledges that Gray and Buechler do not disclose producing a human antibody display library using populations of nucleic acids encoding human immunoglobulins from transgenic mice (final office action at p. 4, second paragraph). Kucherlapati and Lonberg are cited as disclosing transgenic animals expressing human immunoglobulins (final office action at pp. 5-6). Kucherlapati is further cited as teaching combination of phage display technology with such a transgenic animal (final office action at p. 7). The Examiner takes the view it would have been obvious to combine the references for the benefit of producing high affinity antibodies (final office action at p. 7). With respect to previous claim 46 (whose elements are now included in all independent claims), the Examiner takes the view that it would have been obvious and within the skill of the art of the artisan at the effective filing date of the present application to design a set of primers selected based on which human immunoglobulin genes are present in the genome of the transgenic mouse (final office action at pp. 7-8). The Examiner bases this view on the high level of skill in the art, and the teachings of Gray that libraries of high affinity antibodies can be obtained without the use of customized primers (final office action at p. 8).

2. Summary of the Cited Art

Kucherlapati discusses transgenic mice encoding human immunoglobulin genes. All of the examples and most of the general description are directed to isolation of human antibodies from such mice via hybridoma technology (see e.g., pp. 20-31). That is B-cells from such mice are fused with lymphomas to generate hybridomas which secrete human antibodies. Kucherlapati does briefly and prophetically discuss an alternative means of isolating antibodies from such mice using phage display (see pp. 11-12). In this discussion, Kucherlapati does not provide any indication that modifications to previous phage display protocols might be desirable to adapt phage display to use in combination with a transgenic mouse. Thus, Kucherlapati teaches that antibody chains be amplified using the primer set previously used by Marks et al., *J. Mol. Biol. 581-596* (1991) (of record) (see Kucherlapati at p. 13, lines 7-9). The Marks reference uses phage display to screen antibody sequences from an unimmunized human. Marks' primer sets contain far fewer primers than there are natural immunoglobulin genes. Therefore, Marks probably selected his primers either from certain representative immunoglobulin sequences or from consensus sequences of different immunoglobulins. In any event, Mark's primers were not selected based on the subset of human immunoglobulins that are present in a particular transgenic mouse, as specified in the present claims.

Lonberg also discusses transgenic mice encoding human immunoglobulin chains. The reference does not discuss primers for use in cloning populations of nucleic acids encoding human antibodies from transgenic mice.

Buechler and Gray provide essentially the same disclosure relating to improved methods of phage display for isolating populations of high affinity antibodies. The examples in these patents relate to obtaining populations of antibodies from normal (i.e., nontransgenic mice). There is no discussion of primers for cloning populations of nucleic acids encoding human antibodies from transgenic mice.

3. The Prior Art Does Not Teach All Claim Limitations

The prior art references when combined must teach or suggest all of the claim limitations. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Here, even assuming

arguendo that the cited references are properly combined, the references neither individually or in combination provide any disclosure of customized primers, as claimed. The only reference providing any discussion of primers for cloning populations of human antibody genes from transgenic mice is Kucherlapati. The only such primers discussed in this reference are noncustomized primers discussed by Marks. These primers are not designed with respect to a subset of human immunoglobulin genes present in transgenic mice. Rather, these are intended for amplification of immunoglobulin sequences present in an immunized natural human.

The difference between using a primer set containing primers customized to amplify the human immunoglobulin sequences present in a transgenic mouse, and a primer set intended for amplification of immunoglobulin sequences present in an unimmunized natural human is illustrated by the attached two figures (previously of record). The upper part of Figure 1 shows the amino acid sequence from the N-terminus of human heavy chains isolated using customized VH primers as exemplified in the present specification at p.43 (these primers are designated as the Biosite/Medarex primers in the Figures). The sequences designated M1- or M2- are the same as corresponding sequences shows at pp. 84-87 in the present application. The sequences designated 1C- or 3E- are described in commonly owned related application PCT US 00/27237. The lower part of Fig. 1 shows the amino acids encoded by Marks' primer compared with the primers disclosed in the present application. The column labeled "hits" indicates how many of the antibody sequences are encoded by a particular primer. Twenty-nine of thirty-one heavy chain sequences are encoded by one of the primers disclosed in the present application. By contrast, only 13 of the 31 heavy chain sequences are encoded by one of Marks' primers. Use of Marks' primers to attempt to amplify other than these 13 immunoglobulin sequences would either not result in amplification due to lack of complementarity or would result in introduction of mutations. Fig. 2 presents similar data for light chain sequences. In this case, Marks primers encode only 11 of the 31 sequences. Accordingly, use of Marks primer set would result in loss or mutation of a substantial number of antibodies that are obtained using a primer set customized to the human immunoglobulin genes present in a transgenic mouse.

Thus, the selection of customized primers can have a significant effect on the libraries generated by the claimed methods. The prior art does not teach this claim limitation.

4. No Motivation to Modify Cited Art

It is undisputed that the cited art does not teach the claim limitation of customized primers, as discussed above. The sole remaining issue is whether sufficient motivation to modify the cited art has been identified. The motivation must have sufficient "force" to "impel persons skilled in the art to do what applicant has done." *Ex parte Levengood,* 28 USPQ2d 1300, 1302 (BPAI 1993). "Actual evidence" of "clear and particular" motivation is required. *In re Dembiczak*, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999). "Broad conclusory statements regarding the teaching of multiple references" are insufficient. The requirement for evidence of particularized motivation provides a safeguard against the "tempting but forbidden zone of hindsight." *Dembiczak* at p. 1616. Here, several potential sources of motivation asserted by the Examiner to alter the teaching of the cited references will be considered in turn.

The first alleged source of motivation is that "applicants failed to provide any objective evidence for why one of ordinary skilled artisan would limit exclusively the teachings of Kucherlapati et al. with the use of Marks' set of primers" (office action of November 12, 2002 at sentence bridging pp. 14-15). The Examiner also says that Kucherlapati does not teach the exclusive use of Marks' primer set, such as the mouse of Lonberg (final office action at p. 12). Insofar as the Examiner looks to appellants to identify negative teaching in the reference regarding using primers other than Marks, he is incorrectly transferring the PTO's burden of proof to appellants. In proceedings before the Patent and Trademark Office, the examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art (*In re Piasecki*, 223 USPQ 785, 787-88 (Fed. Cir. 1984)). If the evidence is in "equipoise," an inventor is "entitled to a patent." *In re Oetiker,* 24 USPQ2d 1443, 1447 (Fed. Cir. 1992) (Plager, J., concurring). Although teaching away evidence may be sufficient for patentability, it is not necessary. The burden is on the Examiner to show that the reference discloses or suggests use of the customized primer sets specified in the pending claims, not for appellants to identify negative or teaching away evidence. Here, Marks' primer sets are the only primers mentioned by Kucherlapati. Kucherlapati does not provide any reason that one would want to consider any other primers, either with his own mice or with any other, such as that of Lonberg. Kucherlapati provides no indication even that this issue is worthy of further consideration. In these circumstances, pointing to lack of teaching away evidence does not fulfill the PTO's burden of

providing actual evidence of clear and particular motivation to modify the Kucherlapati's teaching to select Marks's primers.

Next the Examiner alleges that it "would have been obvious and within the level of skill for an ordinary artisan to devise an appropriate customized primer set for PCR amplifying the genes encoding high-affinity antibodies depending on which transgenic mouse being used." The Examiner adds the level of skill in the art is high and that the artisan can think. Final office action at p. 12. However, "[t]hat which is within the capabilities of one skilled in the art is not synonymous with obviousness." *Ex parte Gerlach,* 212 USPQ 471 (Bd.App. 1980). An "assertion that one of ordinary skill in the relevant art would have been able to arrive at applicant's invention because he had the necessary skills to carry out the requisite process steps" is an "inappropriate standard for obviousness." *Orthokinetics Inc. vs. Safety Travel Chairs Inc.*, 1 USPQ2d 1081 (Fed. Cir. 1986). "The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggested the desirability of the modification." *In re Fritch,* 23 USPQ2d 1780, 1784 (Fed. Cir. 1992). Here, the cited art does not suggest the desirability of the modification. The desirability is only apparent from the type of analysis discussed in section VIII 3. above. Kucherlapati provides only a brief and prophetic discussion of combining phage display and transgenic mice. The reference directs the reader to a set of primers that had been used previously for amplifying human immunoglobulin libraries for phage display. He provides no reason to look to other primers or even any indication that this issue was worthy of further consideration. Although, as the Examiner says, the skilled artisan can think, he is "*presumed to be one who thinks along the lines of conventional wisdom in the art....*" *Standard Oil Co. vs. American Cyanamid Co.*,227 USPQ 293, 398 (Fed. Cir. 1985), at p. 454 (emphasis supplied). Kucherlapti's teaching to use the primers of Marks, which were conventional in the art, would merely have reinforced this mindset.

Next the Examiner points to Gray or Buechler as demonstrating feasibility of obtaining libraries of high affinity antibodies without use of customized primers (final office action at p. 12). Initially, it is noted that Gray and Buechler amplified libraries of mouse antibodies from nontransgenic mice, and do not specify primers for use in cloning human antibodies from a transgenic mouse. Insofar as Gray or Buechler achieved libraries with high

affinities without use of customized primers, and such teaching was thought relevant to producing libraries of human antibodies from transgenic mice, then such disclosure would teach away from rather than toward the use of customized primers. If one thought that previously used primer sets could not be improved on, one would not have been motivated to consider different strategies of primer design.

Finally, the Examiner says the claims do not recite any characteristics of the customized primers that yield unexpected results as asserted by appellants. However, appellants' position is not dependent on a showing of unexpected results. Rather, appellants' position is that that the Examiner has not made a prima facie case showing that the prior art references when combined teach or suggest all of the claim limitations. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

Further, it is submitted that the cited references did not provide a reasonable expectation of success of obtaining the population of at least 100 human antibodies at least 50% of which have an affinity of at least 10^9 M^{-1} , as recited in claim 1, and particularly not the libraries of at least 100 human antibodies at least 50% of which have an affinity of at least 10^{10} M^{-1} recited in claims 40 and 44. The prior art cannot be modified or combined to reject claims as prima facie obvious without a reasonable expectation of success. *In re Merck & Co, Inc.*, 231 USPQ 375, 379 (Fed. Cir. 1986). Here, the feasibility of generating the claimed libraries is evidenced by the Table at p. 75 showing affinities in that the 10^{10}-10^{11} M^{-1} range, and p. 65, lines 19-29 showing that a high proportion of antibodies that were tested have such affinities. The success of the presently claimed methods in providing virtually unlimited numbers of high affinity human antibodies was not reasonably expected viewed from the perspective that generation of human antibodies with high affinity has long been regarded as a difficult task.

For most of the prosecution up to the final office action, the Examiner's principal basis that the references provide a reasonable expectation of success has been Kucherlapati's alleged teaching that combination of the Xenomouse with phage display offers significant advantage over previous applications of phage display (see e.g., final office action at sentence bridging pp. 5-6, office action of November 12, 2002 at p. 13, second paragraph)). However, the Examiner has taken this statement out of context. Although Kucherlapati does indicate that combination of phage display with the Xenomouse may be advantageous over previous

applications of phage display, the advantage he identifies is that of extending the application of phage display to generation of human antibodies to human antigens (pp. 12-13).

As Kucherlapati explains, phage display has been successfully used by others such as Burton et al. to generate moderate affinity antibodies (ca. 10^8 M^{-1}) to nonhuman antigens such as HIV, but has been much less successful in generating human antibodies to human antigens because of the inability to use such antigens as immunogens in a human. According to Kucherlapati, use of the Xenomouse would allow one to immunize with a human antigen, and thereby presumably use phage display to obtain human antibodies to the human antigen in similar fashion to that employed by Burton to generate human antibodies to HIV. Kucherlapati does not say, however, that combination of the Xenomouse with phage display would allow one to generate higher affinity human antibodies to a human antigen than one could generate to a nonhuman antigen without a Xenomouse, such as described by Burton. The Examiner is thus taking Kucherlapati's comment on the advantage of combining phage display with the Xenomouse out of the context in which it was made, and overgeneralizing it into a general advantage of antibodies prepared using a combination of phage display and the Xenomouse to antibodies made using the Xenomouse alone.

The Examiner also refers to Table 4 of Kucherlapati apparently as disclosing examples of the high affinity human antibodies that might be generated by the combination of phage display and a transgenic mouse (office action of November 12, 2002 at p. 13, second paragraph). However, the Examiner ignores the fact that the antibodies referred to in Table 4 were not generated by a combination of phage display and a transgenic mouse but using a transgenic mouse alone. As discussed in the last response, the natural pairings of heavy and light chain which are represented in antibodies isolated directly from a Xenomouse are likely to be lost during phage display. Thus, antibodies isolated using a combination of phage display and a Xenomouse would not necessarily be expected to have similar affinities to those isolated from the Xenomouse directly. In addition, Kucherlapati provides no indication of how many antibodies he had to screen to obtain the few high affinity antibodies shown in Table 4 of Kucherlapati. Thus, it is not at all apparent that Kucherlapati was able to isolate high affinity human antibodies at high frequency directly from the Xenomouse in contrast to the presently claimed methods.

For these reasons, it is not reasonably predictable from the Kurcherlapati reference that one could combine phage display and a transgenic mouse to generate the claimed libraries displaying large populations of high affinity human antibodies.

The Examiner also refers to a claim in Lonberg as specifying a human antibody produced from a transgenic mouse with an affinity of 10^{10} M^{-1} (final office action at p. 6, second paragraph) However, it is not disputed that antibodies having such affinities can be produced directly from transgenic mice. What is at issue is the frequency of representation of such antibodies both as directly isolated from a transgenic mouse, and when isolated by combining phage display with a transgenic mouse when the additional variable of random assortment of heavy and light chains occurs. As was noted in connection with Table 4 of Kucherlapati, Kucherlapati does not indicate the representation of high affinity human antibodies obtainable directly from a transgenic mouse, much less whether this representation is maintained notwithstanding the effects of random assortment of heavy and light chains. The Examiner has not identified what teaching in Lonberg compensates for this deficiency in Kucherlapati.

In the final office action, the Examiner discounts the above arguments on the basis that Gray or Buechler rather than Kucherlapati is the primary reference (final office action at p. 10). The Examiner even questions whether appellants are implying that the patent of Buechler is invalid (final office action at p. 11, second paragraph). In response, appellants certainly do not imply that Buechler's patent is invalid, but merely point out that neither Buechler nor Gray provides data on the affinity of human antibodies that can be isolated from a transgenic mouse using phage display or otherwise. Indeed, the Examiner himself has stated that "regarding to claims directed to a library in which library members encode heavy and light human antibody chains having specific affinity at least $10^{9}M^{-1}$ and $10^{10}M^{-1}$, neither reference [Gray or Buechler] clearly demonstrates that such a library was obtainable" (office action of November 12, 2002 at p. 6, second paragraph). In the circumstances, it is evident, notwithstanding the protestations to the contrary in the final office action, that the Examiner is relying primarily on Kucherlapati for establish a reasonable expectation of success. Kucherlapati does not do so for the reasons discussed above.

IX. CONCLUSION

The Examiner has not identified actual evidence of clear and particular motivation to modify the teachings of the cited references to use a set of primers selected based on which human immunoglobulin genes from the full complement of human immunoglobulin genes are present in a transgenic mouse. Alleged lack of teaching away evidence in Kucherlapati, the level of skill in the art, and broad conclusory statements regarding the other cited references cannot compensate for the absence of an evidentiary source of particularized motivation. Absent the safeguard provided by an evidentiary source of particularized motivation, one has no way of knowing that the mental reconstruction needed by the Examiner to obtain the claimed invention was not the result of hindsight. Moreover, the Examiner has not established the cited art provided a reasonable expectation of success. For these reasons, it is respectfully submitted that the outstanding rejection should be reversed.

Respectfully submitted,

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APPENDIX A: PENDING CLAIMS

1. A method of producing a human antibody display library, comprising:

providing a transgenic mouse whose genome comprises a plurality of human immunoglobulin genes that can be expressed to produce a plurality of human antibodies, wherein the transgenic mouse comprises less than the full complement of human immunoglobulin genes present in a human being;

isolating a population of nucleic acids encoding human antibody chains from lymphatic cells of the transgenic mouse by amplifying the population of nucleic acids using a set of primers selected based on which human immunoglobulin genes from the full complement of human immunoglobulin genes are present in the genome of the transgenic mouse;

forming a library of display packages displaying the antibody chains, wherein a library member comprises a nucleic acid encoding an antibody chain, and the antibody chain is displayed from the package, wherein the library comprises at least 100 members at least 50% of which comprise nucleic acids encoding human antibody chains showing at least 10^9 M^{-1} affinity for the same target and no library member constitutes more than 50% of the library.

2. The method of claim 1, further comprising producing RNA transcripts of the nucleic acids, and translating the transcripts to form antibody chains under conditions in which an antibody chain remains linked to the RNA transcript from which the antibody chain was translated, the complex formed between the transcript and the antibody chain constituting a library member.

3. The method of claim 1, further comprising cloning the population of nucleic acids into multiple copies of a phage display vector and expressing the vector in host cells to form the library of display packages.

4. The method of claim1, wherein the display package comprises a phagemid vector.

5. The method of claim 1, wherein the nucleic acids encode variable regions of the antibody chains and the display package comprises a segment encoding a human constant region

and the cloning joins a nucleic acid encoding a variable region in-frame with the segment encoding the human constant region.

6. The method of claim 5, wherein the antibody chain is a heavy chain and the constant region comprises a C_H1 region.

7. The method of claim 5, wherein the antibody chain is a light chain and the constant region comprises a C_κ or C_λ constant region.

8. The method of claim1, wherein the antibody chain comprises a heavy or light chain which in at least some library members is complexed to a binding partner, comprising respectively a partner light or heavy human chain to form a Fab fragment.

9. The method of claim 1, further comprising contacting libraries members with a target, whereby library members displaying an antibody chain and binding partner (if present) with specific affinity for the target bind to the target, and separating display packages displaying antibody chains bound to the target to produce a subpopulation of display packages.

10. The method of claim 9, further comprising immunizing the transgenic mouse with an antigen.

11. The method of claim 10, wherein the antigen is the target or an immunogenic fragment thereof.

12. The method of claim 1, wherein a library member further comprises a nucleic acid segment encoding a tag linked to the nucleic acid encoding the antibody chain, wherein the tag is the same in different library members.

13. The method of claim 12, further comprising contacting library members with a receptor having specific affinity for the tag and isolating a subpopulation of library members that bind to immobilized receptor.

14. The method of claim 13, further comprising contacting the subpopulation of library members with a target lacking specific affinity for the tag, and isolating a further subpopulation of library members that binds to the target.

15. The method of claim 14, further comprising subcloning en masse nucleic acids encoding antibody chains from the further subpopulation of library members into multiple copies of an expression vector to form modified expression vectors.

16. The method of claim 15, further comprising expressing the modified expression vectors in host cells to produce a library of human antibody chains.

17. A method of producing a human Fab phage display library, comprising:

providing a transgenic mouse whose genome comprises a plurality of human immunoglobulin genes that can be expressed to produce a plurality of human antibodies, wherein the transgenic mouse comprises less than the full complement of human immunoglobulin genes present in a human being;

isolating populations of nucleic acids respectively encoding human antibody heavy chains and human antibody light chains from lymphatic cells of the transgenic mouse by amplifying the populations of nucleic acids using a set of primers selected based on which human immunoglobulin genes from the full complement of human immunoglobulin genes are present in the genome of the transgenic mouse;

cloning the populations into multiple copies of a phage display vector to produce a display library, wherein a library member comprises a phage capable of displaying from its outersurface a fusion protein comprising a phage coat protein, a human antibody light chain or human antibody heavy chain, wherein in at least some members, the human antibody heavy or light chain is complexed with a partner human antibody heavy or light chain, the complex forming a Fab fragment to be screened, wherein the library comprises at least 100 members at least 50% of which comprise nucleic acids encoding Fab fragments showing at least 10^9 M^{-1} affinity for the same target and no library member constitutes more than 50% of the library.

18. The method of claim 17, wherein the plurality of human genes is free of human lambda light chain genes.

19. The method of claim 17, wherein there are no more than 40 human VH genes included in the plurality of human genes.

20. The method of claim 17, wherein there are no more than 40 human VL genes included in the plurality of human genes.

21. The method of claim 17, wherein each copy of the phage display vector receives a random combination of nucleic acids encoding heavy and light chains from the respective populations.

22. The method of claim 17, wherein the populations of nucleic acids respectively encode populations of human heavy and light chain variable regions, and the phage display vector encodes human heavy and light chain constant regions expressed in frame with human heavy and light chains inserted into the vector.

23. The method of claim 17, further comprising contacting libraries members from the display library with a target, whereby library members displaying a Fab fragment with specific affinity for the target bind to the target, and separating phage displaying Fab fragments bound to the target to produce a further subpopulation of phage.

24. The method of claim 23, further comprising isolating a phage displaying a Fab fragment that binds to the target.

25. The method of claim 17, further comprising immunizing the transgenic mouse with an antigen.

26. The method of claim 24, further comprising expressing a Fab fragment from a phage bound to the target in soluble form.

27. The method of claim 17, wherein the fusion protein further comprises a tag that is the same in different library members.

28. The method of claim 27, further comprising contacting library members with a receptor that specifically binds to the tag, and isolating a subpopulation of library members bound to the receptor.

29. The method of claim 28, further comprising contacting the subpopulation of library members with a target lacking specific affinity for the tag, and isolating a further subpopulation of library members bound to the target.

30. The method of claim 29, further comprising subcloning a mixed population of nucleic acids encoding human antibody heavy chains and human antibody light chains from the further subpopulation of library members into multiple copies of an expression vector to produce modified expression vectors.

31. The method of claim 30, further comprising expressing the modified expression vectors in host cells to produce a population of human antibodies.

32. The method of claim 31, wherein the population of human antibodies includes at least 10 different antibodies.

33. The method of claim 32, wherein the population of human antibodies includes at least 100 different antibodies.

34. The method of claim 33, wherein the population of human antibodies includes at least 1000 different antibodies.

Figure: 1 Compilation of Human Heavy amino termini amplified with the Biosite/Medarex PCR primer set and compared with the Marks' human V_H Back Primers (Marks et.al. 1991).

	1				50	Primer
1CB1H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTLR	SYAMHWVRQA	PGKGLEWVAV	948
1CC2H	EVQLVQSGGG	VVQPGRSLRL	SCAASEFTFS	NYAFHWVRQA	PGKGLEWVAI	946
1CC6H	QVQLVQSGGG	VVQSGRSLRL	SCAASGITVR	NYAMHWVRQV	PGKGLEWVAV	944/1a
1CC8H	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	NYAFHWVRQA	PGKGLEWVAI	944/1a
1CD7H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFS	NYAMHWVRQA	PGKGLEWVAI	948
1CE8	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	NYAFHWVRQA	PGKGLEWVAI	944/1a
3E1H	EVQLVQSGGG	LVQPGGSLRL	SCAASGFTFS	NYAMSWVRQA	PGKGLEWVSA	946
3E2H	QVQLVQSGAE	VKKPGESLKI	SCKGSGYSFT	NYWIGWVRQM	PGKGLEWMGF	944/1a
3E3H	QVQLVQSGAE	VKKPGESLKI	SCKGSGYSFT	NYWIGWVRQM	PGKGLEWMGF	944/1a
3E4H	QVQLVQSGGG	VVQSGRSLRL	SCAASGITVR	NYAMHWVRQV	PGKGLEWVAV	944/1a
3E8H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFR	RYGMHWVRQA	PGKGLEWVAV	948
3E9H	QVQLVQSGAE	VKKPGESLKI	SCKGSGYSFT	NYWIGWVRQM	PGKGLEWMGI	944/1a
M1_10H	QVQLVQSGGG	LVHPGGSLRL	SCEGSGFIFR	NHPIHWVRQA	PGKGLEWVSV	944/1a
M1_1H	QVQLVESGGG	VVQPGKSLRL	SCAASEFTIS	YYGMHWVRQV	PGKGLEWVAA	948
M1_21H	QVQLVQSGGG	VVQPGKSLRL	SCAASGFTFS	YYGMHWVRQV	PGKGLEWVAA	944/1a
M1_23H	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	NYGMHWVRQA	PGKGLEWVAA	944/1a
M1_25H	QVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	YYGMHWVRQV	PGKGLEWVAA	948
M1_3H	DVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVTL	?
M1_4H	QVQLVESGGG	VVQPGKSLRL	SCAASGFTFS	YYGMHWVRQV	PGKGLEWVAA	948
M1_5H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVTL	948
M1_8H	QVQLVQSGGG	VVQPGKSLKL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVAA	944/1a
M2_11H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVTL	948
M2_12H	DVQLVESGGG	VVHPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWMTL	?
M2_16H	QVQLVQSGGG	VVQPGKSLRL	SCAASGFSLS	YYGMHWVRQV	PGKGLEWVAA	944/1a
M2_18H	QVQLVQSGGG	VVQPGKSLRL	SCAASGFSFS	YYGMHWVRQV	PGKGLEWVAA	944/1a
M2_20H	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWVSL	944/1a
M2_31H	QVQLVESGGV	VVQPGRSLRL	SCAASGFTFS	YYGIHWVRQV	PGKGLEWVAL	948
M2_32H	QVQLVQSGGG	LVHPGGSLRL	SCEGSGFIFR	NHPIHWVRQA	PGKGLEWVSV	944/1a
M2_33H	QVQLVQSGGG	VVQPGRSLRL	SCAASGFTFS	YYGMHWVRQA	PGKGLEWMTL	944/1a
M2_34H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTFS	YYGIHWVRQV	PGKGLEWVVL	948
M2_35H	QVQLVESGGG	VVQPGRSLRL	SCAASGFTIS	YYGIHWVRQV	PGKGLEWVEL	948

Marks Human V_H Back Primers

	Amino Terminus	#Hits
$HuV_H1aBACK$	QVQLVQSG	16
$HuV_H2Aback$	QVNLRESG	0
$HuV_H3Aback$	EVQLVESG	0
$HuV_H4Aback$	QVQLQESG	0
$HuV_H5Aback$	EVQLLQSA	0
$HuV_H6Aback$	QVQLQQSG	0

Biosite/Medarex V_H HuMab Primers

	Amino Terminus	#Hits	
#944	QVQLVQSG	16	1aBACK = #944
#945	EVQLLESG	0	
#188	EVQLVESG	0	3aBACK = #188
#946	EVQLVQSG	2	
#947	QVQLQQWG	0	
#948	QVQLVESG	11	

*Bold primers are unique to either Marks'or Biosite/Medarex

Figure 2: Compilation of Human Kappa amino termini amplified with the Biosite/Medarex PCR primer set and compared with Marks' Human V_K Back Primers

	1									50	Primer
1CB1K	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**A**	**T**	**935**
1CC2K	**E**	**L**	**V**	**M**	**T**	**Q**	**S**	**P**	**A**	**T**	**?**
1CC6K	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**G**	**T**	**935**
1CC8K	E	I	V	L	T	Q	S	P	G	T	189/937/3a/6a
1CD7K	E	L	V	M	T	Q	S	P	A	T	?
1CE8K	E	L	V	M	T	Q	T	P	L	S	?
3E1K	E	L	V	M	T	Q	T	P	L	S	?
3E2K	**N**	**I**	**Q**	**M**	**T**	**Q**	**S**	**P**	**S**	**S**	**932**
3E3K	**D**	**I**	**Q**	**M**	**I**	**Q**	**S**	**P**	**S**	**S**	**955**
3E4K	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**G**	**T**	**935**
3E8K	**A**	**I**	**Q**	**L**	**T**	**Q**	**S**	**P**	**S**	**S**	**934**
3E9K	E	L	V	M	T	Q	S	P	S	S	?
M1_10L	D	V	V	M	T	Q	S	P	A	T	936/2a
M1_1L	E	I	V	L	T	Q	S	P	A	T	189/937/3a/6a
M1_21L	**A**	**I**	**R**	**M**	**T**	**Q**	**S**	**P**	**S**	**F**	**933**
M1_23L	E	I	V	L	T	Q	S	P	G	T	189/937/3a/6a
M1_25L	E	I	V	L	T	Q	S	P	G	T	189/937/3a/6a
M1_3L	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**A**	**T**	**935**
M1_4L	E	I	V	L	T	Q	S	P	G	T	189/937/3a/6a
M1_5L	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**G**	**T**	**935**
M1_8L	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**G**	**T**	**935**
M2_11L	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**G**	**T**	**935**
M2_12L	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**G**	**T**	**935**
M2_16L	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**G**	**T**	**935**
M2_18L	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**G**	**T**	**935**
M2_20L	**E**	**I**	**V**	**M**	**T**	**Q**	**S**	**P**	**G**	**T**	**935**
M2_31L	E	I	V	L	T	Q	S	P	A	T	189/937/3a/6a
M2_32L	E	I	V	L	T	Q	S	P	A	T	189/937/3a/6a
M2_33L	E	I	V	L	T	Q	S	P	G	T	189/937/3a/6a
M2_34L	E	I	V	L	T	Q	S	P	A	T	189/937/3a/6a
M2_35L	E	I	V	L	T	Q	S	P	A	T	189/937/3a/6a

Marks Human V_K Back Primers

	Amino Terminus	#Hits
$HuV_K1aBACK$	DIQMTQSP	0
$HuV_K2Aback$	DVVMTQSP	1
$HuV_K3Aback$	EIVLTQSP	10
$HuV_K4Aback$	DIVMTQSP	0
$HuV_K5Aback$	ETTLTQSP	0
$HuV_K6Aback$	EIVLTQSP	(10)

Biosite/Medarex V_K HuMab Primers

	Amino Terminus	#Hits	
#955	DIQMIQSP	1	
#936	DVVMTQSP	1	**(2aBACK=936)**
#189/937	EIVLTQSP	10	**(3aBACK=189/937)**
#931	VIWMTQSP	0	
#932	NIQMTQSP	1	
#937/189	EIVLTQSP	(10)	**(6aBACK=937/189)**
#933	AIRMTQSP	1	
#934	AIQLTQSP	1	
#935	EIVMTQSP	11	
#956	DIVMTQTP	0	

*Bold primers are unique to either Marks or Biosite/Medarex

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